FURTHER NOTES ON THE COCOONS OF ALLOLOBOPHORA FŒTIDA.

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The fact that cocoons of *Allolobophora* are formed during copulation challenges the interpretation that spermatozoa are stored in the spermathecæ in order to produce cross fertilization. We found, however, such conclusive evidence that the spermatozoa of the cocoon are derived from the spermathecæ, that this apparent inconsistency was stated in an earlier paper as a problem for further investigation.[1]

During the summer of 1901, we were able to make at least one step in this direction. We found that cocoons can be formed and deposited when the worms are not copulating. Isolated worms deposited cocoons for weeks, as many as ten cocoons being laid by one worm without copulation.

The method of experimenting was as follows: Single worms with a pronounced clitellum were isolated in half-pint bowls, each bowl being half filled with compost in which the worms had been found. Immediately after this compost was collected, it was carefully sifted to get rid of even the smallest worms, and it was then kept for three or more days before using. This was done to allow any cocoon already in the compost to reach a stage of development that would make it impossible to mistake it for a cocoon laid by the worm isolated for the experiment. At the end of three days the isolated worm was put in a bowl of fresh compost and the compost in which the worm had been kept was sifted under running water through a sieve with meshes too fine to allow a cocoon to pass. By this process nearly all the earth was washed away, and any cocoons present, were easily secured.

A perfectly fresh cocoon, *i. e.*, one with a slime-tube, was often found, but more often the cocoons contained eggs in a later

[1] The Cocoons and Eggs of *Allolobophora fœtida*, Jour. Morph., Vol. XIV., No. 3, 1898, pp. 496, 497.

stage of development, 2, 3, 4, and even 10 or 12 cells. When the stage of any egg appeared doubtful, it was fixed, stained and mounted for examination. Any cocoons in the compost before it was put in the bowls would have had a start in development of at least three days — so it was practically impossible to mistake them for the cocoons laid by the isolated worm.[1]

Twenty worms were isolated for the experiments described, and of these, one worm deposited ten cocoons at average intervals of three days. This worm was vivisected six days after depositing the tenth cocoon, and all four spermathecæ were found to be about one quarter full. The clitellum — which was very pronounced when the worm was isolated — had entirely disappeared, and the seminal vesicles appeared abnormal — they were a brownish-yellow, and quite different in appearance and texture from those of worms captured in the compost heap. This pathological condition, undoubtedly due to confinement, may have prevented the deposition of cocoons continuing until the contents of the spemathecæ was exhausted.

Of the remaining nineteen worms, one deposited four cocoons, and one deposited three, within fifteen days ; one worm deposited three in nineteen days ; two deposited two within seven days, and two deposited two within eight days. Three worms deposited each one cocoon on the third day, one worm deposited one on the fourth day, and one deposited one on the fifth day. In some of these cases the experiment was cut short by the escape of the worm.

Seven of the nineteen worms deposited no cocoon, vivisection showing, however, that two of them were pathological, and that the spermathecæ of four of them were almost empty. We found the spermathecæ full, and the seminal vesicles normal in only one of these worms, and there was no apparent cause why this worm should not deposit cocoons. These worms were isolated from thirteen to twenty days before vivisection.

The experiments described above establish the fact that a

[1] In order to become perfectly familiar with the different stages of development possible within three days, we preserved a large number of fresh cocoons, opening them at intervals of three hours, and fixing, staining and mounting the eggs.

cocoon can be deposited by one worm alone, and our earlier observation of the formation of cocoons during copulation, seems to prove conclusively that there are two methods of forming cocoons. These observations reconcile the opposing opinions[1] on this point.

That the deposition of cocoons by a single worm was not due to isolation, we determined by finding, in pots containing one

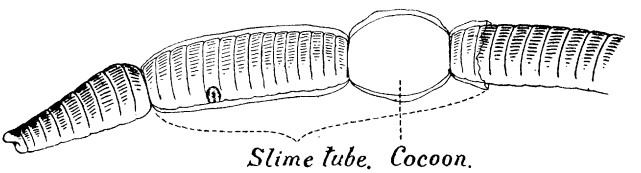

FIG. 1. × 3. Forty-two anterior segments of a worm with a cocoon in the process of formation. The transparent slime-tube surrounds the worm from the tenth to the thirty-sixth segment, and shows constrictions at each edge of the clitellum. The white cocoon encircles the clitellum. When the worm was dropped into boiling water, the cocoon became opaque — effacing the segments of the clitellum.

hundred worms, a number of single worms each with a cocoon around the clitellum. During the summers of 1901 and 1902, we found sixteen of these worms, with cocoons in different stages of development, ranging from a cocoon containing only a trace of albumen and no eggs to one with the full amount of albumen of a mature cocoon, with 28 eggs. These immature cocoons, when removed from the worm, are surrounded by a single slime-tube, resembling one half the double tube found around a copulating pair. This single cocoon lies near the posterior end of the slime-tube (Fig. 3), maintaining the relation that exists between the slime-tube and cocoons in the case of copulating worms, where the two cocoons lie at opposite ends of the double tube. Examination with a lens shows the clitellum of the worm encased by the cocoon and part of the slime-tube, the longer part of the tube lying anterior to the clitellum (Fig. 1). When the worm is not disturbed it will withdraw backward, through the slime-tube and cocoon, finally pulling the head through the smaller end of the cocoon (Figs. 2 and 3). This accords with

[1] We have not been able to find any record other than ours of actual observation of the deposition of cocoons by copulating pairs, or of single worms with cocoons in process of formation.

our observation of copulating pairs, for in these cases also, the longer part of the slime-tube lies anterior to the clitellum, each worm finally withdrawing backward through the small end of the cocoons. In the worm shown in Fig. 1, the slime-tube extends to the tenth segment, but it is not safe to assume that in the living worm the tube may not extend still further, possibly covering the spermathecal openings between the ninth and tenth, and tenth and eleventh segments. The sudden

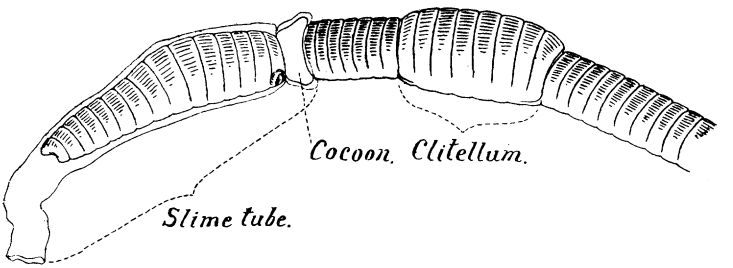

FIG. 2. × 3. Forty-two anterior segments of a worm caught with a nearly mature cocoon around its clitellum. Before being dropped into boiling water, the worm partly withdrew from the cocoon, leaving it encircling the 16th–18th segments. The cocoon and segments are very much contracted, the cocoon appearing as a narrow, opaque band around the worm. Such cocoons regain the typical oblong shape when removed from the worm. The worm has partly withdrawn from the slime-tube, leaving the anterior end projecting beyond its head.

extension of the worm, when dropped into boiling water, possibly causes such change in the position of the slime-tube, as shown in Fig. 1.

On the other hand, we have no proof that the contractions of the worm when captured do not result in an extension of the tube beyond the point covered under normal conditions, and that in the living worm the tube does not reach the spermathecal segments. In living worms, we have seen the slime-tube, in some cases, stop short of these segments, in others, completely cover them, but it is impossible to affirm which condition prevails when the worm is at rest. Fig. 3 shows a tube with its posterior limit clearly indicated, by the impress of five segments posterior to the clitellum. A comparison with Fig. 1 shows that the slime-tube in the captured worm stops short of this point.

Our method of securing cocoons in the process of formation

on single worms, was to dump daily several pots, each containing one hundred worms and carefully to examine the clitellum of each worm. The presence of a slime-tube, with or without a cocoon, can be detected, by constrictions at each end of the clitellum. These constrictions are very marked when the worm is contracted and difficult to see when the worm stretches out. When the worm succeeds in partially escaping from the slime-tube, one or both constrictions can be seen on, or near, the clitellum. These constrictions are produced by the slime-tube and

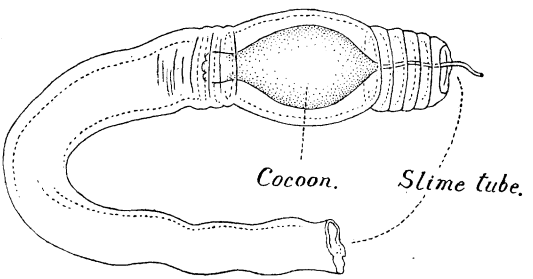

FIG. 3. A freshly deposited cocoon. When on the worm the shorter end of the slime-tube is posterior to the clitellum and the impress of 5 segments on the tube shows that, in this case, the posterior end of the slime-tube extented to the 39th segment of the worm. This figure was reduced one fourth from Fig. 3 of an earlier paper. The original figure was magnified seven diameters.

not by the cocoon, for we have found worms showing marked constrictions, when careful examination of the slime-tube after removal, failed to show any trace of a cocoon. This indicates that the formation of the slime-tube is the first step in the process of forming a cocoon.

Having established the fact that cocoons are formed by these worms, during copulation or separately, the opposing opinions on this point are harmonized. The question now remains, which method predominates?

We find eggs in the cocoon while it still encircles the clitellum, proving that they must be conveyed through the slime-tube, from the oviducts on the 14th segment, to the cocoon; and that they are not deposited in the cocoon when it finally passes over these segments, as suggested by several investigators.

We have found from one to twenty-eight eggs generally near the anterior end of the cocoon. In some cases these cocoons

were so little advanced in development that without a lens they could not be detected until the slime-tube was removed from the worm. Examination of the tube revealed an immature cocoon, with a white semi-transparent covering, containing a much smaller quantity of albumen than is found in mature cocoons, but the eggs showed the stage of development found in a freshly deposited cocoon, *i. e.*, the first maturation spindle at the metaphase and in the center of the egg. Evidently the delicate covering of the cocoon is formed before the albumen is deposited, but the substance that forms the chiton of the covering of the older cocoons is not present, for we have preserved these immature coverings in moist earth for twenty-four hours, and they failed to become yellow and tough, like the covering of the mature cocoon under these conditions; they were white and soft at the end of the experiment.[1] When the slime-tube is torn from these immature cocoons, we find the covering of the cocoon consists of two layers; an outer, perfectly transparent layer, that can be slipped off the inner covering, which is thinner, more resistant, and semi-transparent. The former appears to be part of the slime-tube substance that adheres to the cocoon, but in later stages, when the cocoon is ready for deposition, this layer if preserved becomes yellow like the under layer, which indicates it to be part of the covering of the mature cocoon.

Spermathecæ.

In 10 per cent. of a large number of worms dissected, each with a pronounced clitellum, all four spermathecæ were found empty, this proving that the appearance of the clitellum does not indicate whether the spermathecæ are full or empty, and also that cocoons can be formed until the spermathecæ are entirely empty. In a few cases we found the spermathecæ of copulating worms empty, even when several spermatophores were present in the slime-tube over the spermathecal segments, showing that, spermatozoa are not received into the spermathecæ during the early stages of copulation.

The condition of the individual spermathecæ varies greatly. Sometimes we found only one of the four entirely empty, and

[1] In only one case, an immature cocoon, with a trace of albumen and one egg, showed this characteristic yellow tinge after three hours in water.

again only one full, while the contents of the other three varied in amount. We were unable to discover any constancy in these relations.

Spermatophores.

Having often observed the deposition of cocoons by copulating worms, one of us[1] was led to conclude that the spermatophores in the double slime-tube represented the first step in the formation of the two cocoons. If this were true, we should find spermatozoa in immature cocoons at the earliest stages. The albumen from the immature cocoons found around the clitellum of single worms was dried on slides, stained with iron hæmatoxylin and examined under a Zeiss 4-mm. and a 2-mm. immersion lens, with the aid of a mechanical stage. This albumen resembled that found in freshly deposited cocoons, but *did not contain* spermatozoa. It is evident from the above, that the spermatozoa are put into the cocoon at a later period, possibly when the cocoon passes over the spermathecal openings as it is deposited. The fact, however, that the slime-tube shown in Fig. 1 extends to the posterior spermathecal openings suggests that in its normal position the tube *may* extend to enclose all four spermathecal openings, and the spermatozoa like the eggs be conveyed through the tube to the cocoon, before deposition. But the albumen of three cocoons apparently just ready for deposition failed to show any spermatozoa. These cocoons tightly encircled the clitellum and were equal in size to a freshly deposited cocoon; one contained eleven eggs, two normal; one contained twelve eggs, six normal; and one contained twenty-eight eggs, five normal. The white covering of one cocoon after one hour; of one after twenty minutes; and of one after ten minutes, in water, became tough and yellow like the fresh cocoon a few minutes after deposition.

That spermatozoa are not found in nearly mature cocoons, and that spermatophores *are* found in the slime-tube of copulating worms before the covering of a cocoon is formed, proves beyond question that the spermatophore is not a device for conveying spermatozoa into the cocoon. It would seem rather to be a device for filling the spermathecæ, and it is not formed *after* the spermatozoa leave the spermathecæ, as conjectured in an earlier paper.[2]

[1] *Loc. cit.*

[2] *Loc. cit.*, p. 498.

Spermatophores, however, are certainly not received into the spermathecæ in the form of spermatophores, for we have vivisected many copulating worms having spermatophores at each end of the slime-tube (*i. e.*, over the spermathecal openings of both worms) and the contents of the spermathecæ, in every case, was quite unlike the spermatophore substance; the former mixing freely with water, while the spermatophores do not disintegrate even after twenty-four hours in water.

The granular substance secreted by the spermathecal glands[1] which forms the center of the spermatophores also fails to disintegrate in water, and we have not been able to identify this substance in the spermathecæ.

When copulating worms are allowed to withdraw from the double slime-tube, we sometimes find aggregations of spermatophores in the definite areas of the slime-tube where the two cocoons are formed. The constrictions of the slime-tube that close each end of the cocoons, confine the spermatophores to these areas, and when filled with spermatophores, these areas bear such a striking resemblance in form and color to freshly deposited cocoons, that in our earlier observations we misinterpreted them as partly formed, or nearly completed cocoons. The true "partly formed cocoon" has a white semi-transparent covering, contains more or less albumen and several eggs. Prior to 1901 we had found these cocoons only in the compost not around the worm. We found only a few, and in every case the cocoon contained eggs, but as we did not preserve the albumen, the absence of spermatozoa was not discovered and we supposed these cocoons to represent a later stage of development than the aggregations of spermatophores in the slime-tube.

The marked difference in structure of the spermatophore substance and the contents of these cocoons was noted, as well as the fact that in freshly deposited cocoons we found only a very small number of spermatozoa, in comparison with the large quantity found in a slime tube containing spermatophores. It was these inconsistencies that prompted us to undertake the experiments described above.

[1] *Ibid.*, p. 496.